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July 25, 2003

Dr. William S. Stokes, Director
NICEATM NIEHS
P. O. Box 12233
MD EC-17
Research Triangle Park, NC, 27709

Subject: Pending submission to ICCVAM

Dear Dr. Stokes:

We are sending this communication at the recommendation of Jerry Heindel. It is to alert you and the ICCVAM Scientific Advisory Committee of the intention of IA, Inc./ThreeFold Sensors L.L.C. to submit pre-validation data obtained using our EndoTect™ biosensor for detection of estrogen mimics. It is our hope that the instrument, reagents and methods will be accepted by ICCVAM for inter-laboratory validation testing. We anticipate that the prevalidation data will be complete and ready for review by ICCVAM by June, 2004 at the earliest and November at the latest. Below is a brief description of the nature of the pending submission.

The EndoTect™ biosensor for measurement of estrogen mimicking activity has been developed through a collaboration between IA, Inc., Ann Arbor, MI, and Dr. James L. Wittliff of the University of Louisville. Over the course of several SBIR grants obtained through the NIH and the DOD, IA, Inc. has developed a unique portable (8"x8"x2.5") evanescent fiber optic biosensor instrument and associated sensor cartridges. Through work on grants from NIEHS #R44 ES07471 and R44 ES10076, Dr. Wittliff has developed a lyophilized full length recombinant human estrogen receptor alpha (hERα) reagent which is labeled with a near IR fluorophore for use with the fiber optic sensor instrument; and IA,Inc. has developed sensor cartridges of two types.

The first cartridge type has a weak estrogenic ligand on the surface. This cartridge is used to identify the presence of compounds which bind to hERα by virtue of competition with the sensor for binding to fluorophore-labeled hERα. The second cartridge type has the nucleotide sequence of the consensus region of the vitellogenin ERE on the sensor surface. It is used to assess the impact a test sample has upon binding between hER and ERE. Thus the sensor can be used to answer the questions:

- Does the test sample bind to hERα?
- Is it capable of altering transactivation of genes containing EREs?

Use of a single concentration of sample provides a rapid means for assessing samples of water or extracted soil or food for the presence of estrogenic compounds. The estrogenic potency of the sample is expressed as the concentration of estradiol-17β which produces an equivalent sensor response.

If several concentrations of the compound are used, the kinetic constants for interaction are calculated using those of estradiol-17β as a reference for sensor data interpretation.

The sensor instrument provides real time data by virtue of an evanescent field which scans the surface of the fiber. This field extend only about 1000 angstroms from the sensor surface, thus unbound fluorescence is not excited by the evanescent field because it is too far away. Only fluorescence bound to the sensor surface is excited and recorded by the sensor using this innovative technology. This eliminates the need to separate the bound fluorescence from unbound fluorescence before recording data, thereby permitting analysis based upon binding and dissociation rates rather than on the more traditional equilibrium value.

We look forward to advice and guidance from your office as we proceed toward assessment of validation status by ICCVAM.

Sincerely,

Judith L. Erb
CEO of IA, Inc./ThreeFold Sensors